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Glomus mume and *Kuklospora spinosa*: two new species of *Glomeromycota* from China

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Abstract — Two new arbuscular mycorrhizal fungal species, *Glomus mume* (*Glomerales*) and *Kuklospora spinosa* (*Diversisporales*), were isolated from rhizosphere soil of *Prunus mume* in China. *Glomus mume* resembles *G. taiwanense*, but differs by its spore walls swelling to a globose to subglobose structure in or near the middle of the spore apex. *Kuklospora spinosa* resembles *K. colombiana* but differs by the fine spines covering the middle-layer of the outer spore wall.

Key words —morphotaxonomy, Guizhou, Szechwan, HMAS

Introduction

During a survey of arbuscular mycorrhizal fungi associated with *Prunus mume* (Siebold) Siebold & Zucc. (*Rosaceae*) in China, spores of an undescribed species of *Glomus* Tul. & C. Tul. were found in the soil samples collected from the Maolan National Nature Reserve (25°14′–25°18′N 107°56′–108°10′E) of subtropical woodland in southeast Guizhou province. A second undescribed species, representing *Kuklospora* Oehl & Sieverd., was found in the soil samples collected from the brushy mountain of Muli county (27°58′N 101°15′E) in Szechwan province. The two arbuscular mycorrhizal fungi are described herein as *Glomus mume* and *Kuklospora spinosa*, based on spore morphology.

Materials & methods

Soils were collected from the rhizosphere of *Prunus mume*, from 5–20 cm soil depth. The soil samples of ca. 1000 g were placed in sterilized cotton-cloth bags, labeled, and

† Prof. Jun-yu Chen, a senior member of Chinese Academy of Engineering, died on 8 June 2012.

air-dried in shade. Then they were cleaned from plant debris, sieved with a 2 mm sieve, stored at 4°C, until processed.

Spores were isolated according to Gerdemann & Nicolson (1963) with the wet sieving and decanting method as modified by Daniels & Skipper (1982). Arbuscular mycorrhizal fungal spores were identified following the descriptions of Schenck & Pérez (1988), Morton & Redecker (2001), and more recent species descriptions of *Glomeromycota*. We follow the classification proposed and summarized by Oehl et al (2011). Colors were referred to http://invam.caf.wvu.edu/otherinfo/articles/colorchart.jpg, the INVAM color chart. Specimens were deposited at the Herbarium Mycologicum Academiae Sinicae (HMAS) in Beijing.

Taxonomy

Glomus mume B.P. Cai, Jun Y. Chen, Q.X. Zhang & L.D. Guo, **sp. nov.** Plate 1:A–C

Fungal Name 570036

Differs from *Glomus taiwanense* by its spore walls swelling to a globose to subglobose structure, in or near the middle of the spore apex.

Type: China, Guizhou, Libo, Maolan National Nature Reserve, from the rhizosphere of *Prunus mume*, 17 Sep. 2005 (**Holotype**, HMAS143348).

Etymology: Referring to the plant species with which this fungus was originally associated.

Sporocarps yellow-brown (20–20–20–0) to dark brown (0–30–40–0), globose to subglobose, 350–600 × 380–650 µm, with spores formed radially in a single, tightly packed layer around a central plexus of hyphae. Peridium absent. Spores clavate, cylindro-clavate, triangular or irregular, 90–165 × 30–50 µm, tapering to a cylindric subtending hypha 6–12 µm. Chlamydospore wall laminate, yellow-brown (20–20–20–0) to dark brown (0–30–40–0), 1.5–4 µm thick on the side walls, swelling to a globose to subglobose structure, 13–20 × 15–25 µm, in or near the middle of the spore apex, and thickened to 3–7 µm at spore base. Spore base at hyphal attachment with a small pore, open or occluded by wall thickening. Reaction to Melzer's reagent not distinctive.

Ecology — Spores of *Glomus mume* were isolated from soil sampled from the rhizosphere of *Prunus mume* growing at 700–800 m a.s.l in the karst forest area of southwest China (25°19′N 107°57′E). The chemical properties of the soil sample were: pH 6.62, organic matter 146.27 $g.kg^{-1}$, total N 6.5 $g.kg^{-1}$, organic N 0.51 $g.kg^{-1}$, total P 0.48 $g.kg^{-1}$, organic P 0.025 $g.kg^{-1}$, and exchangeable K 0.13 $g.kg^{-1}$.

Glomeralean species occurring with *G. mume* were *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüssler, *Funneliformis geosporus* (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler, *Glomus australe* (Berk.) S.M. Berch, *G. clavisporum* (Trappe) R.T. Almeida & N.C. Schenck, *G. multicaule* Gerd. & B.K. Bakshi, *G. rubiforme* (Gerd. & Trappe) R.T. Almeida & N.C. Schenck, and *Septoglomus deserticola* (Trappe et al.) G.A. Silva et al.

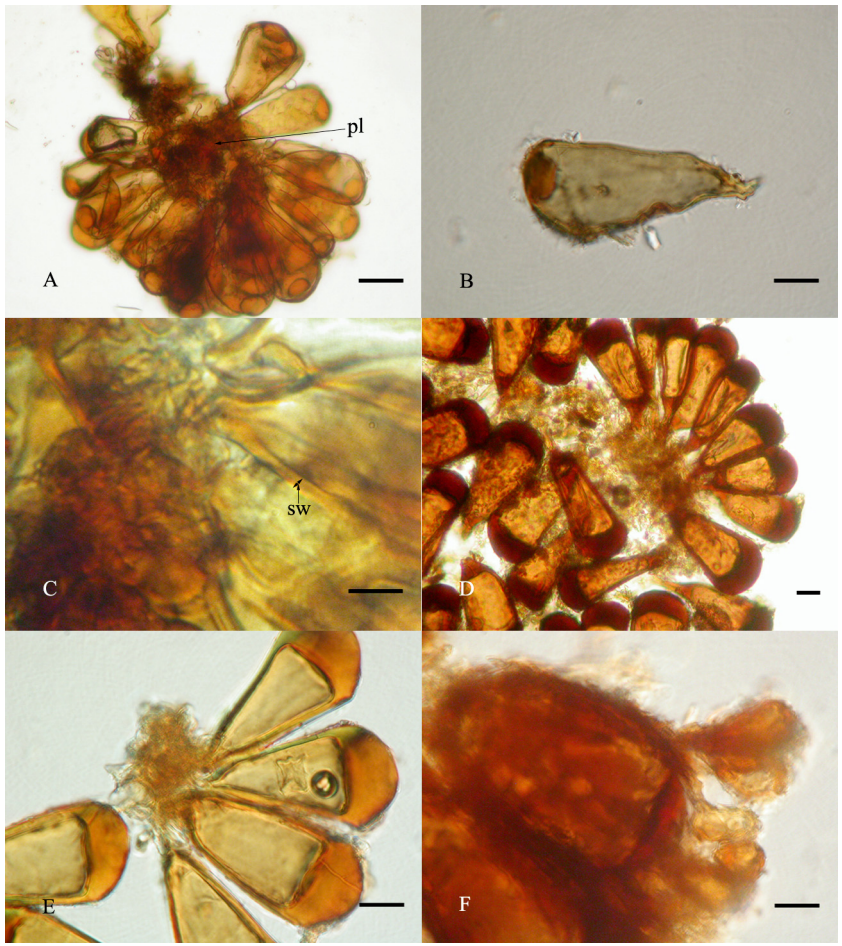

Plate 1. *Glomus mume* and morphologically similar species. ***Glomus mume***: A. Broken sporocarp showing the central hyphal plexus (pl) in Melzer's reagent; B. Spore in Melzer's reagent; C. Structure of the spore wall (sw) in Melzer's reagent. ***Glomus clavisporum***: D. Portion of the sporocarp. ***Glomus taiwanense*** E. Portion of the sporocarp. ***Glomus liquidambaris***: F. Spores with "paraphysis-like" structures. Scale bars: A, B, D, E, F = 20 µm; C = 10 µm.

Kuklospora spinosa B.P. Cai, Jun Y. Chen, Q.X. Zhang & L.D. Guo **sp. nov.** Plate 2

Fungal Name 570037

Differs from *Kuklospora colombiana* by fine spines covering the middle-layer of the outer spore wall.

Type: China, Szechwan, Muli country, from the rhizosphere of *Prunus mume*, 5 Sep. 2005 (**Holotype**, HMAS142950).

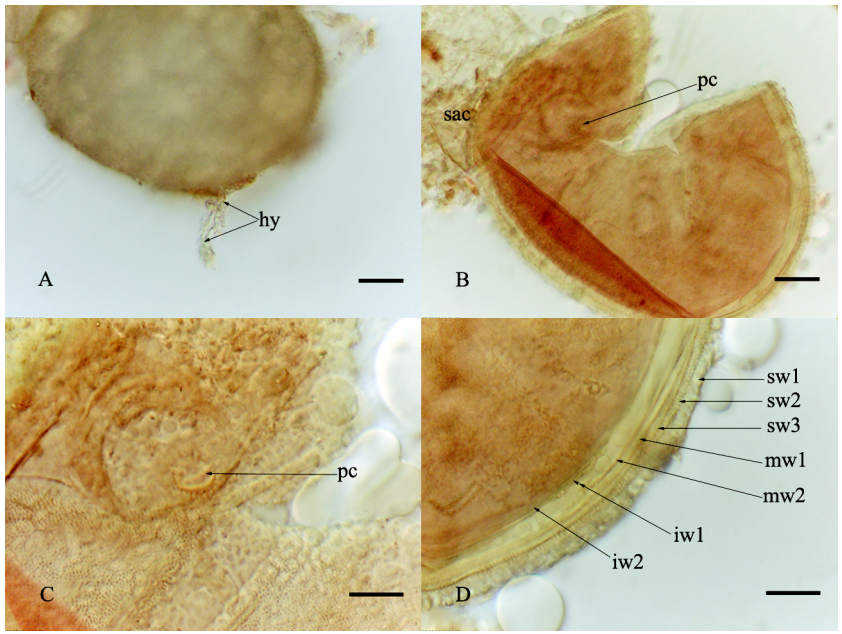

Plate 2. *Kuklospora spinosa*. A. Spore with a distal hypha (hy) in water; B. Spore connections to the sporiferous saccule (sac) and the cicatrix proximal to saccule (PC) in Melzer's reagent; C. Cicatrix proximal to saccule (PC) in Melzer's reagent; D. Broken spore with three-layered outer wall (sw1–3), two layers of the middle wall (mw1 & mw2) and the inner wall (iw1 & iw2); iw2 stained by Melzer's reagent. Scale bars: A, B = 20 μm; C, D = 10 μm.

Etymology: Latin, *spinosa*, referring to the ornamentation on the surface of the outer laminate spore wall layer (sw2).

Sporocarps unknown. Spores formed by inflating the hyphal stalk in some distance to a terminally or intercalary formed sporiferous saccule. Spores hyaline, subhyaline to yellowish-white (0–10–20–0), globose to subglobose, 100–250 μm diam. Spores have three walls: an outer spore wall (sw), a middle wall (mw) and an inner wall (iw). The outer wall (sw) tri-layered (sw1, sw2 and sw3): sw1 hyaline, 1–1.5 μm thick, evanescent, usually completely degraded or sloughed in mature spores; sw2 laminate, yellowish-white (0–10–20–0), 3–5 μm thick, covered with fine spines, 2–3 high × 0.5 μm in diam, which grow into sw1; sw3 hyaline, 0.5–1 μm thick, tightly adherent to sw2, usually separated from sw2 in Melzer's reagent, reaction to Melzer's reagent not distinctive. The middle wall (mw) hyaline, bilayered (mw1 and mw2), thin, less than 1 μm thick, no reaction in Melzer's reagent. The inner wall (iw) bilayered (iw1 and iw2): iw1 membranous, granular, beaded, 0.5–1 μm thick, granules sometimes scattering in crushed spores; iw2 plastic, 0.5–1 μm thick, staining pink (0–30–20–0) to

reddish purple (0–40–60–10) in Melzer's reagent. Sporiferous saccule hyaline, globose, 90–150 µm diam, collapsing at maturity and leaving a cicatrix proximal to saccule and a distal hypha or cicatrix. Cicatrix proximal to saccule, 8–15 µm diam, distal hypha, 1.5–3 µm wide at the spore base. Wall of soporiferous saccule bilayered, 1.5–3 µm thick.

Ecology — Spores of *Kuklospora spinosa* were isolated from soil sampled under *Prunus mume* growing at 2450–3100 m a.s.l. in the brushy mountain of southwest China (27°58′N 101°15′E), The chemical properties of the soil sample from which *G. mume* was isolated were: pH 6.48, organic matter 56.32 $g.kg^{-1}$, total N 2.5 $g.kg^{-1}$, organic N 0.15 $g.kg^{-1}$, total P 0.48 $g.kg^{-1}$, organic P 0.037 $g.kg^{-1}$, and exchangeable K 0.2 $g.kg^{-1}$.

Glomeralean species occurring in the same samples were *Acaulospora tuberculata* Janos & Trappe, *Claroideoglomus claroideum* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüssler, *C. luteum* (L.J. Kenn. et al.) C. Walker & A. Schüssler, *Diversispora trimurales* (Koske & Halvorson) C. Walker & A. Schüssler, *Funneliformis geosporus*, *F. mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler, *Glomus clavisporum*, and *Septoglomus deserticola.*

Discussion

Glomus mume is morphologically similar to *G. clavisporum*, *G. taiwanense* (C.G. Wu & Z.C. Chen) Y.J. Yao, *G. liquidambaris* (C.G. Wu & Z.C. Chen) Y.J. Yao, and *G. coremioides* (Berk. & Broome) D. Redecker & J.B. Morton. The sporocarpic spores of *G. mume* can be easily separated from these four species by the swelling in the middle of the spore apex (Plate 1A, B). The spore wall of *G. clavisporum* is 1–1.5 µm thick at the sides, thickened to 17–25 µm at the apex and to 5–8 µm at the base (Wu & Chen, 1987; Almeida & Schenck, 1990; Plate 1D). Spore wall of *G. taiwanense* is 1–1.5 µm thick at the sides, thickens to 4–25 µm at the apex and to 1–5.5 µm at the base (Wu & Chen, 1987; Almeida & Schenck, 1990; Plate 1E). Spore wall of *G. liquidambaris* is 2–5 µm thick at the sides, thickens to 7.5–25 µm at the apex and to 6–10 µm at the base, and the prominent characteristic of this latter species is a "paraphysis-like" structure protruding from the central plexus of hyphae to the outer part of the sporocarp (Wu & Chen, 1987; Almeida & Schenck, 1990; Plate 1F). Spore wall of G. *coremioides* is 2–2.5 µm thick, up to 4 µm thick near the spore base (Almeida & Schenck, 1990).

Kuklospora was erected by Sieverding & Oehl (2006), to accommodate two species previously described in *Entrophospora: K. colombiana* (Spain & N.C. Schenck) Oehl & Sieverd. and *K. kentinensis* (C.G. Wu & Y.S. Liu) Oehl & Sieverd. *Kuklospora spinosa* has a morphology typical for the genus based on the formation of spores within the stalk of the sporiferous saccule and the presence of a beaded inner wall layer (iw1). Spores of *K. spinosa* differ clearly

from those of *K. colombiana* and *K. kentinensis* in size, colour, and outer spore wall ornamentation.

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